Medical Student Writing Award Winner

Chronic Fatigue Syndrome
A Critical Appraisal of the Role of Epstein-Barr Virus

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Editor

The symptom complex currently designated the chronic fatigue syndrome was previously termed the chronic or chronic active Epstein-Barr virus syndrome or the chronic mononucleosis syndrome, prematurely assuming an etiologic role for the Epstein-Barr virus (EBV). This presumption derived from the fact that some patients with the chronic fatigue syndrome have very high or very low titers of certain antibodies to EBV. A review of seroepidemiologic patterns of response to EBV and of studies of patients with the chronic fatigue syndrome shows that these antibody titers overlap considerably both with those of controls or other healthy persons and with those of patients with other illnesses. Given the high prevalence of exposure to EBV, it would be difficult to determine whether the virus caused the syndrome or whether the antibody elevations resulted from the illness, even if distinct differences in titers existed. Other methodologic issues of control selection, laboratory test comparability, and differing case definitions pose problems in studying this syndrome. The recently published working case definition should facilitate the continuing search for causes.

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The chronic fatigue syndrome encompasses a myriad of definitions that include many nonspecific symptoms. Persistent disabling fatigue lasting at least 1 to 18 months prevails as a constant feature of all definitions. Variably incorporated into the syndrome are fever, pharyngitis, myalgias, arthralgias, headaches, paresthesias, lymphadenopathy, and psychologic or neurologic complaints. Similar syndromes in the past include Iceland disease, epidemic neuromyasthenia, encephalomyelitis, and the postviral syndrome.[1-5] As yet the causes of these syndromes remain unknown.

The resemblance of the chronic fatigue syndrome to a chronic or recurrent form of infectious mononucleosis has provoked investigation of a possible link between the syndrome and the Epstein-Barr virus (EBV), the etiologic agent of infectious mononucleosis. Early studies found presumptive evidence in some patients of persistent EBV infection, as shown by high titers of immunoglobulin (Ig) G antibodies to Epstein-Barr viral capsid or early antigens or a lack of the expected antibodies to EBV-determined nuclear antigens.[6-9] On this basis, despite the fact that healthy persons may have similar titers, some investigators termed the syndrome the chronic mononucleosis syndrome, or the chronic active EBV syndrome, implying that EBV is the cause. To demonstrate the problems with this assumption, I will review briefly the seroepidemiology of the Epstein-Barr virus.

Seroepidemiology of Epstein-Barr Virus

Determining the pattern of antibody response both during acute disease and many years later is necessary to evaluate a possible causative role for EBV in the chronic fatigue syndrome. Comparing the types of antibodies prevalent in both the healthy and the ill—including those with the chronic fatigue syndrome and patients with cancer, immunosuppression, and other diseases—will clarify this issue further.

Based on studies of acute infectious mononucleosis, it is generally thought that during primary infection with the Epstein-Barr virus, IgM against the viral capsid antigen (VCA) and antibody to the early antigens—especially those distributed diffusely throughout the nucleus and cytoplasm, early antigen (EA)-D—appear early, rise in titer, and then decline to undetectable levels, the IgM to VCA after a few weeks, the anti-EA antibodies after a few months.[10-13] The presence of IgG to VCA and antibody against the Epstein-Barr nuclear antigen (EBNA) persists indefinitely,[10,13] although anti-EBNA usually does not develop until several weeks or months after the acute illness.[11] The prevalence of IgG anti-VCA depends on the age and the socioeconomic status of the group sampled, increasing with increasing age and with decreasing socioeconomic status[14-17]; approximately 90% of adults in the United States show serologic evidence of a previous EBV infection. Many questions remain to be answered, however. For example, how long does each type of

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ABBREVIATIONS USED IN TEXT

AIDS = acquired immunodeficiency syndrome
CMV = cytomegalovirus
EA = early antigen
EBNA = Epstein-Barr [virus] nuclear antigen
EBV = Epstein-Barr virus
Ig = immunoglobulin
VCA = viral capsid antigen

antibody persist? Are there variations in the level of antibodies and in antibody response patterns, and, if so, do certain patterns signify "healthier" responses? What composes the range of normal antibody responses?

Several studies of healthy people have attempted to answer these questions. In a prospective study, Niederman and co-workers found EBV antibody at levels of 1:40 or more at least four years after an episode of infectious mononucleosis and up to seven years after asymptomatic seroconversion.[17] Horwitz and associates observed 88 patients for 10 to 104 months after EBV antibody-confirmed infectious mononucleosis.[18] Titers of IgG to VCA remained high (≥1:320) in 56% at 10 to 14 months and in 27% at 40 to 104 months. Anti-EBNA antibody titers ranged from 1:5 to 1:160. In a systematic sample of a semirural community, Sumaya and colleagues found that 98.6% of healthy adults between 31 and 50 years of age had anti-VCA antibody titers of 1:10 or greater[16]; 21% of this age group had titers of 1:160 or greater, and 34% of those older than 50 years had titers as high. Additionally, 100% of healthy pregnant women in two studies showed seropositivity for anti-VCA antibody, with geometric mean titers of 346 and 273, and 95% to 100% were anti-EBNA positive, with geometric mean titers of 43 and 30.[19,20] These studies show the wide range of anti-VCA and anti-EBNA antibody levels found and the duration of their persistence in healthy people.

Because anti-EA is thought essentially to disappear soon after infection, the presence of antibody to the early antigens suggests a recent primary infection, and the presence of IgM to VCA would confirm this fact. Seropositivity instead for anti-EBNA provides evidence for a previous EBV infection. Between 12% and 63% of the patients in the above studies had antibodies to the early antigens, however, with titers of 1:10 or greater, and in another study by Sumaya, 19 of 20 healthy adults tested positive for IgM anti-VCA, anti-EA, and anti-EBNA.[21] The simultaneous presence of all three antibodies may rarely occur after a primary infection,[11] but one would not expect so many persons older than 30 years to have had a recent primary infection. Sumaya suggested that this pattern could be a manifestation of a heterologous antibody response to another virus, but he concluded that it most likely represents a host immune response to an endogenous reactivation of EBV. Given, however, that the persons tested were all healthy and had no history of a mononucleosis-like disease, a definitive conclusion cannot be reached.

Distinct antibody patterns with very high titers of antibodies are commonly observed in EBV-related diseases such as Burkitt's lymphoma and nasopharyngeal carcinoma. African patients with Burkitt's lymphoma have extremely high titers of anti-VCA (87% ≥1:160, geometric mean titer 376), with a variable presence and titers of anti-EBNA; most of them have titers of antibodies to EA—in the restricted cytoplasmic, perinuclear distribution, EA-R—that are significantly higher than in normal controls.[22] A decrease in the titer of anti-EA-R antibodies during remission is predictive of five-year survival. In patients with nasopharyngeal carcinoma, high levels of anti-VCA (86% ≥1:160, geometric mean titer 350) and anti-EA-D antibodies predominate. The presence of IgA to VCA or EA-D is also useful as both a diagnostic and a prognostic tool in cases of nasopharyngeal carcinoma, with titers increasing with higher stages of the disease and decreasing after successful therapy.[22,23]

Interestingly, high titers of anti-VCA antibody also prevail in immunosuppressed patients with malignant or nonmalignant diseases, such as lymphoproliferative disorders or other cancers, sarcoidosis, systemic lupus erythematosus, and immunodeficiency diseases. The antibody titers in these diseases generally do not rise as high as those in patients with Burkitt's lymphoma, nasopharyngeal carcinoma, or infectious mononucleosis. Of 489 patients with Hodgkin's disease, 40% had titers of 1:160 or greater, with a geometric mean titer of 105, and of 235 patients with chronic lymphocytic leukemia, 45% had titers of 1:160 or greater with a geometric mean titer of 110.[22] These patients may also have IgM anti-VCA and low, absent, or normal levels of anti-EBNA.[24] In addition, renal transplant patients often have high levels of anti-VCA, low levels of anti-EA-R, and no anti-EBNA antibody.[23] Thus, serologic evidence of "abnormal" or "reactivated" EBV does not necessarily confirm the presence of disease caused by EBV, for immunosuppressed patients have no illness presumed to be caused by Epstein-Barr virus.

In summary, antibody responses to Epstein-Barr virus show many serologic patterns. Any or all of the EBV antibodies discussed have been found in healthy persons long after a primary infection. Pregnant women, hospital inpatients, and immunosuppressed patients also have a high prevalence of anti-VCA, anti-EA, and anti-EBNA antibodies without apparent clinical evidence of EBV-related disease. Moreover, while the levels of these antibodies generally do not surpass those found in persons with known EBV-related diseases, the high titers exceed those traditionally expected many years after an EBV infection. Reviewing these seroepidemiologic patterns of response to EBV enables a critical evaluation of the etiologic hypothesis relating EBV to the chronic fatigue syndrome.

Chronic Fatigue Syndrome

The term "chronic fatigue syndrome" is vague. This symptom complex comprises fatigue or easy fatigability, malaise, and sometimes pharyngitis, fever, or other symptoms, but it has few signs and no proven cause. Because it often follows a flulike illness and resembles a chronic form of mononucleosis, the syndrome has been more specifically termed the chronic Epstein-Barr virus syndrome, chronic active EBV infection, or the chronic mononucleosis syndrome. The lack of specificity, however, of either the syndrome definition or the symptoms themselves warrants withholding such terminology, especially as the evidence linking EBV to the syndrome is tenuous at best. Despite the problematic definition, investigators currently prefer to use the term chronic fatigue syndrome because it describes the most salient clinical feature of the syndrome and does not imply a particular cause or causes.[25]

The more specific terms usually label syndromes more clearly associated with EBV. Chronic active EBV infection often is used for patients with underlying primary or sec-

ondary immunodeficiency states who have extremely high titers of anti-VCA and anti-EA antibodies and absent or lower titers of anti-EBNA. These patients have extensive disease caused by polyclonal B-cell lymphomas that are positive for the EBV genome or EBNA.[26,27] Recently Jones and co-workers described three patients with chronic recurring disease and objective signs such as hepatosplenomegaly, pancytopenia, weight loss, and extraordinarily high titers of anti-VCA (1:10,000) and anti-EA-D (1:640) and low or normal titers of anti-EBNA.[28] On postmortem examinations they discovered polymorphic T-cell lymphomas positive for the EBV genome. Chronic EBV syndrome also refers to a severe disease with high titers of EBV antibodies (with either high or absent anti-EBNA) and clinical signs such as fever, pneumonitis, and lymphoid hyperplasia and polyclonal hypergammaglobulinemia[29] or pancytopenia,[30] a syndrome unlike the chronic fatigue syndrome.

Descriptive Epidemiology

As reported in recent literature, persons suffering from the chronic fatigue syndrome are mostly white and of a higher socioeconomic status, with twice as many women afflicted as men.[6-9,31] They range in age from 3 to 56 years, with a mean age of approximately 30. Dubois and associates noted no blacks among his patients with the chronic fatigue syndrome, even though blacks constitute half of Dubois's private practice.[7] Of 14 patients in the Dubois study, 9 had college degrees, as did 21 of 23 patients in the study of Straus and colleagues.[7,9]

Many aspects of the chronic fatigue syndrome limit our acceptance of this admittedly sketchy descriptive epidemiology. Because each investigator had his or her own slightly different definition of the syndrome, we cannot be certain that each group of patients has the same disease. In fact, due to the nonspecific case definition, we do not even know that the patients within each study have the same disease with the same cause.

Because these patients were often drawn from physicians' private practices,[9,31] they may not represent well members of the general population with this syndrome. Media publicity may have led some patients to their physicians. Referral bias probably played a role once certain investigators communicated a special interest in the syndrome.[7,9,31] It may be asked whether those of higher socioeconomic classes are more susceptible to this problem or whether they merely have better access to physicians, particularly these physicians, than the poor.

More important, the inclusion by some investigators of "abnormal" EBV findings on serologic tests—IgM anti-VCA, anti-EA, and high IgG titers of anti-VCA[6-8]—in the case definition further limits the representativeness of the patients studied. The ubiquitous prevalence of EBV infection in serologic examinations in the United States population makes it difficult to sort out true and false linkages; including EBV serologic study in the case criteria overestimates EBV linkage with the chronic fatigue syndrome.

Etiologic Studies

Many studies have attempted to confirm the role of EBV in the chronic fatigue syndrome. They have investigated patients with some of the various symptoms, evaluating their EBV antibody status and titers and comparing the percent of positive antibody titers with those expected in normals or found in control populations. Some studies have noted significant differences in antibody status or titers between cases and controls. These values have little diagnostic value not only because the controls are often poorly chosen or ill-defined, but also because the results overlap with both those of the healthy controls and those expected in patients with other illnesses. A lack of uniformity in case definitions and in laboratory standards, the latter both within and between studies, may also blur true distinctions or create artificial differences. A major problem with these studies, even if distinct differences existed, is that they are essentially prevalence studies; therefore, whether EBV caused the fatigue syndrome or the antibody elevations resulted from immunologic activation due to the illness would be difficult to determine.

One of the earlier studies by Tobi and co-workers sought EBV-definitive serologic findings in a case series.[6] In their patient population "derived from a large group . . . in which a viral etiology was suspected," they selected seven who had symptoms for more than a year, no other serious disease, and the presence of IgM anti-VCA for that year. These patients tested negative for rheumatoid factor, which can cause a false-positive test result for IgM anti-VCA. In this group of patients, the authors also found high titers of IgG anti-VCA ($\geq$1:128) and titers of anti-EBNA antibody persistently 1:40 or higher. Four of the seven also tested positive for anti-EA-R ($\geq$1:8). The coincidence of IgM anti-VCA with anti-EBNA is unusual because these antibodies signify distinct temporal relations to primary infection,[13] although as mentioned above such a combination has occurred in asymptomatic persons.[21] Tobi and associates thought that this serologic pattern may indicate a reactivation of EBV, but the selection of only IgM-positive cases and of no IgM-negative cases or IgM-positive controls for comparison makes it difficult to draw any conclusions regarding a causal role for EBV.

Dubois and colleagues studied 14 patients largely from Dubois's private practice in Georgia.[7] These patients were chosen on the basis of chronic fatigue and malaise in association with "serologic findings of active EBV infection." For serologic studies, the investigators used three different laboratories and did not specify which laboratories did which tests, nor did they mention whether the laboratories had comparable results. They used controls largely for reference values, picking 19 female and 12 male hospital personnel, neither age- nor sex-matched to cases. All cases tested positive for IgG to VCA (range of multiple specimens from cases, 1:40 to $\geq$1:1,280), anti-EA, and anti-EBNA, and negative for IgM to VCA. By comparison, none of the controls tested positive for anti-EA, and their titers of IgG generally fell in a lower range (1:40 to 1:320), although they were not compared directly and the actual values are not given. In two patients, anti-EA titers disappeared although the patients remained symptomatic, while another patient continued to have an anti-EA titer but became asymptomatic. Despite the fact that they chose the patients based on their unspecified "serologic evidence of active EBV infection" (perhaps excluding those who were anti-EA-negative), and the fact that anti-EA titers were not fully predictive of symptoms, the authors concluded that "anti-EA is a key serologic marker for CMS (chronic mononucleosis syndrome)."[7]

These patients also differed from the general population, however, in factors for which the investigators did not select. In 12 of 14 patients, cytomegalovirus (CMV) antibody titers

were significantly higher than those of a group of randomly selected blood donors ($P < .001$). Although different laboratories evaluated the CMV antibody titers, the authors noted that at least five patients had evidence of active CMV infection. They also found variable, mild immunoglobulin deficiencies (no numbers given) in 10/14 cases, and in 6 of 7 tested they found slightly decreased T4/T8 ratios—less than one standard deviation below normal. These data could just as easily provide evidence for a cause or for a result of EBV "reactivation."

Jones and co-workers reported on a year-long follow-up of 44 patients aged 3 to 54 referred for evaluation of persistent or recurrent illnesses, with symptoms that included but did not require fatigue, lymphadenopathy, fever, and headaches.[8] Their definition of illness and their inclusion of 18 (41%) children raise the issue of comparability with other studies. Only 39 of these patients tested positive for the EBV antibody, with all 39 positive for IgG anti-VCA and anti-EA. Of 33 age-matched healthy controls from a source or sources not described, 30 tested positive for IgG anti-VCA, but only 3 of the 30 tested anti-EA-positive. Case anti-VCA and anti-EA titers were significantly higher than the respective titers of the controls ($P < .001$). In the graphic representation, however, the ranges of case and control titers overlapped substantially. Jones and colleagues noted a "recognizable" pattern of anti-EA elevation with a recurrence of symptoms in 89% of the patients, but they acknowledged both the presence of anti-EA in healthy persons, including 10% of their seropositive controls, and the possibility that elevated anti-EA levels may follow rather than precede the underlying problem, as a marker of secondary viral "reactivation."

Physicians at the National Institutes of Health and in 11 states referred 31 patients to Straus and associates for their appraisal.[9] These patients gave a history of chronic illness with fatigue lasting at least a year after infectious mononucleosis or a mononucleosis-like disease, and their physicians had declared themselves unable to explain the illnesses. In their article, Straus and co-workers focused on the 23 EBV-seropositive persons. It is important to note that diagnoses initially missed by the patients' primary physicians were finally made in five of the eight seronegative patients. The diagnoses were, respectively, Sjögren's syndrome, systemic lupus erythematosus, poorly differentiated nodular lymphoma, transverse myelitis and Hodgkin's disease, and multiple sclerosis.

They compared the antibody status and titers of the 23 patients with those of 23 age- and sex-matched seropositive controls. As in the article by Jones and associates, the source of the controls was not described. With the use of the highest reciprocal values of those titers drawn from the cases, the geometric mean titer of case IgG anti-VCA was significantly higher than that of the controls (518 versus 71, $P < .001$). These comparisons were problematic, however, because they compared multiple specimens from cases with single specimens taken from controls; 19 of 23 cases had at least some values in the reference range given for "normal seropositives" and 9 of 23 had all values fully within this range. Two different laboratories did the serologic testing; no report is made of their comparability or reproducibility. Other antibody results, with similar methodologic errors, include 19/23 anti-EA-positive cases versus 6/23 anti-EA-positive controls, 16/23 anti-EBNA-positive cases versus 23/23 controls, and 5/23 cases intermittently positive for IgM anti-VCA versus 0 controls. Straus and co-workers discovered that one IgM-positive result was falsely caused by a rheumatoid factor, but the other four results were not reevaluated.[9]

On the basis of the foregoing evidence, Straus and colleagues discussed the possibility of a causal role for EBV in the fatigue syndrome.[9] They cited the following as support: some of the illnesses began with acute infectious mononucleosis; virus-specific antibody levels were elevated, while antibody levels to *Toxoplasma gondii,* CMV, and other viruses were not; and the increased T cell-mediated suppression of immunoglobulin synthesis by EBV- or mitogen-stimulated B cells resembles T-cell activity seen in acute infectious mononucleosis.[32] Although many of the patients' illnesses began with mononucleosis, manifesting a primary EBV infection as infectious mononucleosis could initiate or merely indicate an immune system at risk for superinfection or other disease. As they selected these patients based on their EBV antibody status and many of the titers are within normal range (in addition to overlapping with those of the controls), the antibody results constitute scant evidence for EBV as a cause of the patients' illnesses. Straus and co-workers also pointed out that 7/23 patients lacked anti-EBNA, a defect that has been observed in the immune-deficient persons.[23,24] Together with the suppressor T-cell activity, such data suggest immune dysfunction but give no proof of cause.

Both Miller and colleagues and Henle and associates searched specifically for chronic EBV markers, reporting complementary, equally inconclusive results.[33,34] In patients diagnosed with "chronic active EBV," both groups assessed antibody responses to specific EBNA components. Miller and co-workers discovered that 4 of 39 ($\sim 10\%$) patients lacked anti-EBNA-1 antibodies.[33] Pointing out that 100% of 33 healthy adults, 30 nasopharyngeal cancer patients, and 18 cancer patients have anti-EBNA-1 antibodies, they said that a lack of anti-EBNA-1 may serve as a selective marker for the chronic fatigue syndrome. The lack of sensitivity of such a marker is augmented by a lack of specificity: 80% (12/15) of children with the acquired immunodeficiency syndrome (AIDS) in their study and 10% (4/43) of those with Burkitt's lymphoma also lacked the antibody.

Based on the premise that after infectious mononucleosis, anti-EBNA-2 antibody levels first rise and are later eclipsed by anti-EBNA-1 antibodies, Henle and colleagues measured and compared the levels of these antibodies in healthy and in ill persons. Of 38 healthy seropositive controls, all positive for anti-EBNA-1 antibodies, all had the expected anti-EBNA-1 levels that were greater than anti-EBNA-2 levels. Only 28% (20/70) of patients with the chronic fatigue syndrome had the "abnormal" result, with anti-EBNA-2 levels greater than anti-EBNA-1 levels. In addition to these indeterminate results, the authors remarked that patients with Hodgkin's disease, non-Hodgkin's lymphoma, AIDS, and other immunodeficiency diseases often have similar reversed ratios.

Two studies more rigorously examining this problem were published back to back in 1987. Both Holmes and associates and Buchwald and co-workers carefully delineate the origin and comparability of their study patient populations with their pools of patients, in addition to detailing more completely their methods.[31,35]

Holmes and colleagues interviewed by phone 134 of 139 patients who had been tested serologically for EBV in Nevada. These patients, suffering from fatigue, originated from

an office practice set in the well-to-do resort community of Incline Village. Attempting to make the type of illness studied as uniform as possible, they defined case-patients as those with "persistently increased fatigue lasting at least one month . . . sufficient to cause absence from work for two weeks or longer or reduction of daily activity by 50% or more, with no apparent explanation." Only 15 patients fit this definition; of the rest, "non-case patients," 101 had fatigue that resolved in less than a month or was not as severe and 18 had other possible reasons for fatigue. They compared serologic values of the cases with those of both non-case-patients and 30 age-, sex-, and race-matched controls with no history of fatigue or of EBV testing, 21 of whom were patients from the office practice scheduled for routine laboratory tests, the other 9 being office staff.

They evaluated the antibody data both for potential threshold titers and for differences between geometric mean titers. Generally antibody titers of cases fell above those of either non-case-patients or controls. Significant differences existed at some thresholds and between certain geometric mean titers, but no antibody values could be used to distinguish cases from either of the other two groups because much overlap existed between the groups of values. Among cases and controls, they also compared titers of antibodies to CMV, herpes simplex virus types 1 and 2, and measles, finding that cases tended to show values above those of controls for the other viruses also.

Holmes and co-workers concluded that EBV serologic findings are inadequate for diagnosing the chronic fatigue syndrome and that the evidence is inconclusive regarding an etiologic role for EBV.[31] Having compared the variability of EBV serologic test results both within and between three laboratories, they also decried the subjectivity and the lack of reliability of the test results, a significant point for future studies and for interpreting previous studies. They pointed in addition to the necessity of remaining open to other possible causes of these illnesses.

Buchwald and associates approached the etiologic question from a different angle.[35] Rather than study highly selected patients with the chronic fatigue syndrome, they conducted a prospective study to ascertain the prevalence of the syndrome in a general practice setting and to determine the usefulness of EBV antibody testing in such patients. During a six-month period, they interviewed 500 patients coming to the practice for any reason. Their general patient population was not described, although these 500 were said to be representative of all patients between the ages of 17 and 50 years seen in the practice during this period. Included as cases were patients having both severe loss of energy or easy fatigability for more than six months and either pharyngitis, myalgias, or headaches. Pregnant women and patients receiving steroids or with certain chronic diseases were excluded. Of 103 who fit the case definition, only 40 patients consented to participate in the study. Superficially the investigators found no participation bias, as the nonparticipants did not differ significantly in age, sex, or severity of symptoms.

Buchwald and co-workers chose 40 age- and sex-matched controls from those patients scheduled for routine blood tests.[35] A technician unaware of case or control status tested pairs of specimens for EBV antibodies in parallel. No significant differences were found in comparing cases with controls for the presence of VCA-IgG, anti-EA, anti-EBNA, and IgM anti-VCA, nor for certain threshold titers of these antibodies. The geometric mean titers of cases were not significantly higher than those of controls, although the patients had been divided by certain symptoms into five overlapping groups, decreasing the ability to detect a difference in these smaller-sized cells.

Buchwald and colleagues recognized that their patients, who were not seeking care primarily for their fatigue, perhaps could not be compared with the sick or disabled patients in previous studies.[35] They noted that their patients generally had lower antibody levels, and they also acknowledged the flaws in comparing results from different laboratories. As to the relevance of the investigation of the chronic fatigue syndrome, their patients' fatigue and related symptoms, while perhaps not as severe as others had reported, had caused considerable morbidity for a median duration of 16 months. These authors also cited as their rationale the need for studies more representative of the syndrome as manifested in the general population, not merely in groups of highly selected patients. In their patient population, Buchwald and coworkers found no predictive value in EBV serologic testing and no evidence for a causal role for EBV.[35]

Methodologic Issues

The evaluation of the chronic fatigue syndrome and the possible etiologic role of EBV brings with it methodologic problems. Some of these matters arise from investigators' choices of methods, while others are inherent to the study of this disease.

In fact, whether the syndrome comprises only one disease or many diseases with diverse causes is uncertain. The various overlapping, nonspecific case definitions may represent either a spectrum of one disease or distinct diseases that are difficult to separate clinically, and the lack of a strict case definition undermines the ability of studies, both individually and as a whole, to find any one cause. Therefore, while recognizing that the syndrome may not necessarily comprise a single disease, several investigators have published a working case definition to facilitate further research and to increase the comparability between future etiologic studies.[25]

The case finding in these studies requires that the representativeness of these patients—mostly higher socioeconomic class and EBV antibody-positive—be examined. Many of these patients come from selected, highly referred physicians' practices. Other patients may remain undiagnosed because their physician has not heard of or does not believe in the existence of the syndrome. Other patients with the chronic fatigue syndrome may have little access to the media or to physicians. In addition, the inclusion of EBV-antibody positivity in some case definitions automatically excludes those patients with symptoms but without EBV antibody on serologic tests. The proper methods should involve an evaluation of these latter patients, for selecting the cases by the factor to be assessed invariably will overstate the linkage of EBV with the syndrome. Henle and associates noted that they have data, as yet unpublished, regarding a group of patients with symptoms of the chronic fatigue syndrome but no EBV antibodies.[34]

The choice of controls in these studies raises another methodologic issue. Tobi and colleagues had no controls,[6] while other investigations had poorly matched or incompletely described controls.[7-9] Although in later studies con-

trols were selected more carefully than in the earlier ones,[31,35] these controls still were mostly healthy patients having routine laboratory tests. Perhaps sick patients, with illnesses unrelated to EBV, would serve better as controls, for the chronic illness in patients with the chronic fatigue syndrome might be expected to manifest with immunologic aberrations such as a "reactivation" of EBV or other antibodies; using ill persons as controls would permit a recognition that such an event could occur secondary to other prolonged illnesses. As noted earlier,[13,22] hospital inpatients with illnesses presumed to be unrelated to EBV often have some elevated EBV antibody levels.

Laboratory test comparability becomes an issue in studies that rely heavily on EBV serologic test results. It is difficult to compare results when the studies encompass various definitions of what is a positive outcome—for instance, an anti-EBNA titer of 1:8[13] or 1:40.[6] Even if the same threshold titers are used, the results could not be compared, for no laboratory standards exist. Subjective end points for the tests, the varying quality of reagents used, and disparate technicians' abilities in the different laboratories detract from the reliability and validity of serologic results within and between studies. Laboratory standardization clearly is needed.

Even if these studies had shown a more consistent association of certain EBV antibody titers with the chronic fatigue syndrome, they are essentially prevalence studies, and therefore cause and effect cannot be differentiated. As noted earlier, EBV infection and the presence of EBV antibodies are common; EBV seropositivity alone does not indicate an etiologic role. High titers of antibodies might signify confounding factors and not causality, as such high titers could result from a nonspecific immune activation due to the illness. Persons with illnesses unrelated to EBV and even healthy persons have had equally high titers.

Conclusion

These etiologic studies have found generally higher prevalences or levels of EBV antibodies in patients with the chronic fatigue syndrome than in healthy controls but without any clear distinctions between patients and controls. The patients' antibody titers at times matched those of the controls. Often they fell above or below control values in a given study, but still within the range found for healthy normal persons in studies of EBV serologic patterns.[13,15,16] These prevalences and titers also did not exceed those seen in conditions unrelated to EBV, such as pregnancy, cancers, and immunosuppression. The lack of predictive value for individual serologic patterns emphasizes the lack of specificity of EBV serologic findings for the chronic fatigue syndrome, a conclusion corresponding to that of Holmes, Straus, and others.[25,31,35-37]

In their attempts to find other markers for the syndrome, several studies found nonspecific immunologic dysfunctions in patients with the chronic fatigue syndrome,[7,9,38] evidence for the existence of disease but not for its cause or effect. The presence of antibody to other viruses in the studies by Dubois and Holmes and colleagues[7,31] and the fourfold rise in titers to EBV among seven patients with other viral or tuberculous illnesses in the Tischendorf study[15] raise the question of polyclonal B-cell activation or anamnestic antibody responses. A study of the "postviral fatigue syndrome" noted also a syndrome resembling the chronic fatigue syndrome, with vague immunologic dysfunction, but with a significant prevalence of antibody only to Coxsackie B viruses.[5] Perhaps different viruses are causing a similar syndrome.

The foregoing studies, with their methodologic problems, have found no etiologic role for EBV in the chronic fatigue syndrome, although they did not rule out the possibility that EBV causes some cases of the fatigue syndrome. Straus has suggested that some patients, in particular those with the most "severe" serologic abnormalities and an illness developing after proven infectious mononucleosis, may have a chronic EBV infection.[39] Because exposure to and infection with the virus is so common, however, more informative studies regarding its etiologic role in the syndrome must await further advances in the science of the Epstein-Barr virus. A more complete understanding is needed of the pathophysiology of the events after primary infection, the mechanism of latency and reactivation, and the functions of specific parts of the EBV genome. Whether certain EBV strains could be lytic rather than latent is still unknown. With such knowledge, we might understand whether EBV antibody elevations signify primary or secondary viral reactivation and contribute to morbidity, or whether such elevations are merely an epiphenomenon.

Because of the relative lack of objective findings, some physicians dismiss the chronic fatigue syndrome as a psychoneurotic manifestation. Komaroff argues for an organic basis for this disease or diseases, citing the following reasons: patients have symptoms not seen in other psychoneuroses; some patients have laboratory test abnormalities (other than EBV serology); patients all give the same history of good health abruptly altered by this syndrome; and, the reported occurrence of clusters of disease suggests an infectious or environmental agent.[36] Straus supports the view of an organic cause, noting that the syndrome may represent a "general response to various psychological or physical irritants."[37] He nevertheless declares the need for more information in the area of psychopathology and the potential interaction between the psyche and the immune system.

Finally, physicians and scientists should not neglect other alternatives. Before treating patients with nonspecific, unproven γ-globulin[40] or acyclovir—recently shown in a placebo-controlled trial to be unhelpful in the syndrome[41]—physicians are well advised to recall those patients excluded from the Straus study with diseases that their primary physician had not discovered.[9] An important feature of the recently published working case definition is the extensive list of known diseases that must be ruled out before diagnosing the chronic fatigue syndrome.[25] For epidemiologists and other scientists, suggestions of possible causes of the syndrome include a new virus—human B-cell lymphotropic virus[42,43]—other viruses, other infectious or environmental agents, and even postviral thyroiditis.[44] One or all may cause the chronic fatigue syndrome, but unfortunately none have yet been investigated. The future should produce studies of these and other possible causes of the syndrome.

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